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Evaluating Historical Candidate Genes for Schizophrenia

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Abstract

Prior to the genome-wide association era, candidate gene studies were a major approach in schizophrenia genetics. In this invited review, we consider the current status of 25 historical

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URLs

SzGene database (http://www.szgene.org) obtained in 11/2009 (active updating of SZGene ended in 12/2011). Genomic results available via the PGC (http://pgc.unc.edu), genomic visualization using Ricopili (http://www.broadinstitute.org/mpg/ricopili), and gene-centric data at GeneBook (http://atgu.mgh.harvard.edu/genebook). Alzheimer Disease & Frontotemporal Dementia Mutation Database (http://www.molgen.ua.ac.be).

Author Contributions

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Conflicts of Interest

The authors report no conflicts.

candidate genes for schizophrenia (e.g., *COMT*, *DISC1*, *DTNBP1*, and *NRG1*). The initial study for 24 of these genes explicitly evaluated common variant hypotheses about schizophrenia. Our evaluation included a meta-analysis of the candidate gene literature, incorporation of the results of the largest genomic study yet published for schizophrenia, ratings from informed researchers who have published on these genes, and ratings from 24 schizophrenia geneticists. On the basis of current empirical evidence and mostly consensual assessments of informed opinion, it appears that the historical candidate gene literature did not yield clear insights into the genetic basis of schizophrenia. A likely reason why historical candidate gene studies did not achieve their primary aims is inadequate statistical power. However, the considerable efforts embodied in these early studies unquestionably set the stage for current successes in genomic approaches to schizophrenia.

Keywords

schizophrenia; genetics; candidate gene; review; meta-analysis

Introduction

In this review, we consider the current status of candidate genes for schizophrenia that were prominent in the literature prior to the genome-wide association study (GWAS) era. This review was invited by Prof Julio Licinio, the editor of *Molecular Psychiatry*.

Due to the high heritability of schizophrenia[1], there have been many efforts to discover the causative genetic factors, and candidate gene studies have been a major approach. For example, the SZGene database [2] (obtained 11/2009) listed 1406 candidate gene papers investigating over 700 genes. In these studies, one or more genetic markers in genes hypothesized to be involved in the etiology of schizophrenia were genotyped in cases with schizophrenia and controls. Prior to the advances brought about by the Human Genome Project[3] and the International HapMap Project [4], it was difficult and expensive to genotype a comprehensive list of genetic variants in a genomic region. Investigators thus tended to genotype a few genetic markers in a candidate gene selected based on prevailing theories of the etiology of schizophrenia (e.g., antipsychotic pharmacology) or positional candidate genes from linkage or cytogenetic studies.

The candidate gene strategy had a few notable successes in identifying genetic variation for other complex diseases. Influential examples included replicated associations of Alzheimer's disease with common variation in *APOE* [5] and alcohol dependence with common variants in alcohol metabolic genes. [6] It was thus not unreasonable to hope that similar studies might work for schizophrenia. Realization of this expectation proved difficult. A pattern emerged whereby an initial claim of association for a seemingly plausible, even exciting, candidate gene for schizophrenia was followed by a mixed pattern of non-replications and replication. Thus, candidate gene association studies for schizophrenia became controversial. [7–10]

The goal of this review is to evaluate the current status of historical candidate genes for schizophrenia. The motivation is straight-forward: there are hundreds of papers on these genes, several of these genes have motivated considerable biological experimentation, and

as recent large-scale studies have expanded our knowledge base, it is reasonable to review this topic. Early candidate gene studies evaluated tens of genetic markers in hundreds of subjects and more recent studies conducted genome-wide comparisons of millions of genetic markers in tens of thousands of subjects. The largest published study is of 34,000 cases from the Psychiatric Genomics Consortium, PGC) which identified 108 genome-wide significant loci. [11]

Almost all of these historical candidate gene studies evaluated the role of common variation (one part of a spectrum of variants involved in schizophrenia). Our evaluation includes: (a) meta-analysis of candidate gene studies, (b) PGC schizophrenia mega-analysis results,[11] (c) expert evaluations from researchers on specific candidate genes, and (d) survey ratings from schizophrenia genomics investigators. The latter two approaches are not "scientific" in a strict sense, but rather provide a general guide to the "significance" and "impact" that the earlier findings currently have in the field.

Methods

We selected 25 genes prominent in the pre-GWAS era. The first schizophrenia GWAS appeared in 2007 but, given that many candidate gene association studies were published in 2008, we evaluated candidate gene studies published in calendar year 2008 or earlier. The 25 genes we selected were either featured in reviews of the genetics of schizophrenia [10, 12–14] or were highly studied (≥20 papers recorded in SZGene). The genes and the rationale for being a candidate gene for schizophrenia are given in Table 1. We continued several important assumptions made by virtually all candidate gene studies (see Limitations). First, these studies evaluated "schizophrenia" as a dichotomous entity. Second, as with the primary studies, we assumed that genetic variants act on the gene it was in or near. This assumption is crucial and will be inaccurate for a currently unknown proportion of genetic variants. Third, as discussed below, almost all of these studies evaluated common genetic variation.

We evaluated these 25 historical candidate genes for schizophrenia in four ways. First, we conducted fixed-effects meta-analyses for all genetic markers in these 25 genes using summary data on subjects of European ancestry in the SZGene database. [2] Second, we included PGC results for schizophrenia (9.5 million markers in 34,241 cases, 45,604 controls, and 1,235 trios followed by replication analyses of 263 SNPs in 1,513 cases and 66,236 controls). [11] We report the results for the same SNP appearing in SZGene and for the SNP with the smallest *P*-value in a gene (± 25 kb).

Third, we elicited perspectives from "informed investigators" who had published most extensively on or were the original or firmest proponents of a particular candidate gene in schizophrenia. These individuals were identified using PubMed searches: (*gene* [All Fields] OR "*protein name*" [All Fields]) AND ("schizophrenia" [MeSH Terms] OR "schizophrenia" [All Fields]). Informed investigators were contacted to request their summary judgment of the current status of one particular gene as a genetic risk factor for schizophrenia (1=very unlikely and 5=very likely). Genetic risk could refer to common, uncommon, rare, private, or *de novo* genetic variation. A draft of Table 2 was supplied upon request. Informed

investigators were given the opportunity to include text in the Supplement to explain their rating.

Fourth, we obtained perspectives from “schizophrenia geneticists”. We used principal investigators from the PGC schizophrenia working group[11] as a convenience sample. We obtained responses from 24 investigators for summary judgments using the same rating scheme as for the informed investigators. Many of these investigators study common, uncommon, rare, private, or *de novo* genetic variation.

Results

Table 1 summarizes 25 historically important candidate genes for schizophrenia. For 24 of 25 genes, the initial study conducted genotyping to evaluate the impact of common genetic variation on risk for schizophrenia. Some candidate genes were selected because of rare genetic events (e.g., *COMT*, *PRODH*, and *ZDDHC8* are located in the 22q11 deletion CNV) but the study evaluate common genetic variation rather than rare variation. The *DISC1* study genotyped rare variation in a Scottish pedigree. The key findings for three genes were unimpressive for schizophrenia *per se* but presented somewhat more significant findings for putative endophenotypes (*CHRNA7* and *COMT*) or a broadly inclusive set of psychiatric disorders (*DISC1*). Eleven genes were positional candidates based on genome-wide linkage or structural variation (*CHRNA7*, *COMT*, *DAO*, *DAOA*, *DISC1*, *DTNBP1*, *NOTCH4*, *NRG1*, *PPP3CC*, *PRODH*, and *ZDHHC8*). Eight genes derived from a hypothesis about the etiology of schizophrenia based on pharmacology (*AKT1*, *DRD2*, *DRD3*, *DRD4*, *GRM3*, *HTR2A*, *SLC6A3*, and *SLC6A4*). Six genes were from miscellaneous hypotheses (*APOE*, *BDNF*, *KCNN3*, *MTHFR*, *RGS4*, and *TNF*).

Many of the reported common variant SNP or haplotype relative risks were exceptionally large: often >1.5 and >2 for *DRD3*, *HTR2A*, *MTHFR*, *NRG1*, and *PRODH*. Rigorous control for multiple testing of genetic markers, haplotypes, and/or phenotypes was evident in one study (*ZDHHC8*). None of the *P*-values in the primary studies were genome-wide significant [15] ($P<5\text{x}10^{-8}$), and most were not notable after correction for the number of SNPs genotyped.

Figure 1 shows the number of times that each gene or its protein product co-occurred with schizophrenia in a paper indexed by PubMed per year. This serves as a rough metric for the importance/impact of a gene for the schizophrenia research community. For eight genes, the numbers of studies increased with time (*APOE*, *BDNF*, *CHRNA7*, *COMT*, *DISC1*, *DRD2*, *HTR2A*, and *NRG1*). Four genes peaked and then tapered off (*DAO*, *DAOA*, *DTNBP1*, and *RGS4*). For 13 genes, there have been relatively few reports (*AKT1*, *DRD3*, *DRD4*, *GRM3*, *KCNN3*, *MTHFR*, *NOTCH4*, *PPP3CC*, *PRODH*, *SLC6A3*, *SLC6A4*, *TNF*, and *ZDHHC8*).

Table 2 provides four evaluations for each historical candidate gene for schizophrenia. The first evaluation is a meta-analysis of candidate gene association studies in SZGene published in 2008 or earlier showing the most-studied genetic marker per gene (Table S1 shows results for all markers). We have previously shown that the candidate gene studies in SZGene had small samples and poor coverage of common genetic variation.[16] No finding is near

genome-wide significance [15] ($P<5x10^{-8}$), and all *P*-values for this evaluation fall short by a factor of 10,000 or more. None is notable even on a gene-wise basis (which many would consider inappropriately liberal).

The second evaluation reports the results from the large PGC mega-analysis for schizophrenia. [11] We report results for the same SZGene polymorphisms (if available) plus the smallest *P*-value in the gene (±25 kb). The PGC and SZGene results agree on the absence of evidence of association for most of these genes (21/25). Four genes (*DRD2, GRM3, NOTCH4,* and *TNF*) are genome-wide significant in the PGC analysis, but were not implicated by the SZGene meta-analysis. The lack of association for *NOTCH4* and *TNF* in the candidate gene literature is notable given that these genes are in the major histocompatibility complex (MHC). The MHC contains the most significant association common variant association ($P=3.5x10^{-31}$) and the second largest OR (1.2) for schizophrenia. [11] Due to extensive linkage disequilibrium, the MHC contains thousands of genome-wide significant associations extending over 7 Mb. Indeed, there are over 60 genome-wide significant associations ±25 kb of *NOTCH4* and four ±25 kb of the small *TNF* gene (2.8 kb). [11]

The third evaluation was a rating by informed investigators for 12 genes. These individuals introduced the gene into the literature or had published extensively on it. The informed investigators provide fuller explanations for their rankings in the Supplement. Five genes (*AKT1*, *CHRNA7*, *DISC1*, *DRD2*, and *HTR2A*) were rated as highly likely to be a genetic risk factor for schizophrenia (rating ≥4 on a 1–5 scale). With the exception of *DRD2*, none of the informed ratings ≥4 is supported by empirical results from the older SZGene or the newer PGC mega-analysis.

The fourth evaluation consisted of ratings from 24 schizophrenia geneticists. The distribution of ratings is provided in Figure S2. The mean ratings were ≥4 for only *DRD2* and *GRM3*. The mean ratings were discordant with those from informed investigators for *AKT1*, *CHRNA7*, *DISC1*, and *HTR2A*.

Discussion

Our knowledge of the genetic architecture of schizophrenia – the number of loci, allele frequencies, genotypic relative risks, and modes of action – has grown significantly in the past year. The largest GWAS to date suggests that schizophrenia is associated with many common genetic variants of small effect sizes. [11] Several rare CNVs have genotypic relative risks in the 5–20 range. [17] Rare exonic variants of stronger effect do play a role, but it now appears unlikely that schizophrenia has a genetic architecture dominated by such variants. [18, 19] A direct comparison found that common genetic variation accounted for far more of the variance in liability to schizophrenia than rare copy number variation or rare deleterious exonic variation.[18]

Given the importance of common variation in the etiology of schizophrenia and that 24 of 25 historical candidate genes for schizophrenia explicitly posited and evaluated the role of common variation, it is timely to assess the contributions of this literature to our knowledge

of schizophrenia. With the advantages of hindsight (and noting that the authors of this review conducted many candidate gene studies including two in Table 1 [20, 21]), we offer a number of explanatory hypotheses regarding this literature.

Historical candidate genes for schizophrenia in light of current empirical results

First, it is now clear that historical candidate gene association studies for common genetic variation had grossly inadequate statistical power. For example, a candidate gene study of 1,000 cases and 1,000 controls has 0.03% power [22] to detect a genotypic relative risk (GRR) of 1.15 (assuming a log-additive model, lifetime prevalence=0.007, minor allele frequency=0.3, and $\alpha=5x10^{-8}$). A GRR of 1.15 is large for schizophrenia, and only 10 of 128 SNPs [11] reaching genome-wide significance had GRR > 1.15. When power is so low, the probability that a "significant" finding is a false positive is overwhelming. [23, 24]

Second, the largest GWAS to date had essentially 100% power to identify common genetic variants with GRR > 1.15 (minor allele frequency > 0.10) or GRR > 1.19 (minor allele frequency > 0.05). We can thus exclude common genetic effects akin to those for *APOE* and Alzheimer's disease (i.e., GRR of 3.7 for *APOE**ε4 vs. ε3). [25] We can also conclude that the GRRs reported in many of the 24 common variant studies in Table 1 (often >1.5 and >2 for five genes) are inconsistent with what we now know about GRRs for common variation (Table 2, often for the same genetic marker reported in the initial study). Some common variants in Table 2 (e.g., the *KCNN3* CAG repeat or complex haplotypes in *NRG1*) may not have been well-captured in SNP arrays; however, this criticism is mitigated by the lack of evidence from the SZGene meta-analyses for the same variants.

Third, even before the current generation of large genomic studies for schizophrenia, it was reassuring to note that candidate gene meta-analyses by other authors (Table S2) [26–41] and our SZGene meta-analyses (Table 2) were converging on the null. This is important because the candidate gene literature for common variation in schizophrenia is often believed to be replete with false positive claims: this generality is not supported by meta-analysis.

Fourth, the largest and most carefully conducted schizophrenia common variant association study does not provide empirical support for 21 of the 25 historical candidate genes as genetic risk factors for schizophrenia. [11] Two historical candidate genes (*DRD2* and *GRM3*) have genome-wide significant evidence for common variant association with schizophrenia [11] although the candidate gene literature did not support these associations. Two additional candidate genes (*TNF* and *NOTCH4*) have genome-wide significant associations with schizophrenia. [11] These genes are in the extended MHC, a complex region with high gene density and extensive linkage disequilibrium, and the MHC-schizophrenia association may not implicate these genes. The candidate gene literature missed these associations although these should have been the most accessible common variant findings: the MHC was the first genome-wide significant GWAS signal for schizophrenia [42–44] and 11% of high-quality SNPs (6,570 of 57,891) in the extended MHC region exceeded genome-wide significance. [11] These false negatives from the pre-GWAS era likely resulted from extremely low statistical power and limited genotyping.

Fifth, one historical candidate gene (*DISC1*) studied a rare genetic event, the t(1;11)(q42.1;q14.3) translocation in a Scottish pedigree where the propositus did not have schizophrenia. The genetic linkage results in this pedigree point to a broad phenotype (LOD 7.1 for recurrent major depression, bipolar disorder, or schizophrenia). The status of *DISC1* is controversial despite its entry into the literature nearly 15 years ago [45] (see also a rebuttal [46]). The most critical issue is that no other genetic study has independently implicated *DISC1* (i.e., met contemporary significance thresholds for rare exonic variation, rare CNVs, or common variation). [11, 18, 19, 47, 48] In contrast, many other rare variant associations have genetic replication evidence. For example, early-onset Alzheimer's disease is caused by rare mutations in *APP*, *PSEN1*, and *PSEN2*.[49] Unlike the singular *DISC1* event, these associations are highly compelling as they replicate in many different pedigrees (90 families for *APP*, 405 for *PSEN1*, and 22 for *PSEN2*, see URLs). Similarly, the CNV associations for autism and schizophrenia replicate in large samples worldwide. [17]

In conclusion, the current evidence from large and carefully conducted studies of genetic variation does not support the idea that the historical candidate gene literature led to robust and replicable genetic findings with the capacity to provide insights into the etiology of schizophrenia. Most genes (24 of 25) evaluated common variant hypotheses: the large effect sizes posited by initial studies were not confirmed, and four common variant associations that now meet genome-wide significance were missed. These conclusions have an important qualifier. Knowledge of the genetic basis of schizophrenia is incomplete but rapidly growing. Historically large studies were published in 2014, and considerable expansions of sample sizes for common and rare variant analyses are in progress. Some genes in Table 2 could become notable in the future.

Alternative perspectives on historical candidate genes for schizophrenia

The opinions of experts play a role in science particularly when there are few hard data, and have been important in psychiatry. [50] The prominence of some historical candidate genes for schizophrenia has increased despite a lack of strong support from genetic studies (Figure 1). Thus, we also surveyed opinions on these genes. First, for 12 genes, we obtained ratings from informed investigators (i.e., those who introduced a candidate gene into the literature or who published extensively on it, Table 2). We point readers to the Supplement for further explanations from the informed investigators. The informed investigator ratings agreed with the PGC results for seven genes. For five genes (*AKT1*, *CHRNA7*, *DISC1*, *DRD2*, and *HTR2A*), the informed investigator rating was high (a rating ≥4 on a 1–5 scale). For one of these five (*DRD2*), the PGC results concur. For the remaining four genes, the informed investigator ratings ≥4 were different from empirical results.

Second, we obtained ratings from 24 schizophrenia geneticists. The mean ratings were ≥4 for only *DRD2* and *GRM3*. The mean ratings were inconsistent with those from informed investigators for *AKT1*, *CHRNA7*, *DISC1*, and *HTR2A*. Note that all ratings could incorporate any type of genetic variation. In general, we found that empirical data and opinion agreed for most of the 25 candidate genes, and the discrepancies for *AKT1*, *CHRNA7*, *DISC1*, and *HTR2A* stand out. Several informed investigators address this issue and believe that genetic results that do not meet widely accepted standards for significance

in genetics or which lack replication can be augmented by biological data (Supplement). To this view, biological plausibility can provide salience to chance-level genetic results.

We contend that this "biological validation" argument is weak, subjective, prone to incorrect decisions, and liable to divert downstream research efforts by emphasizing the wrong targets. First, as documented in this paper, biology-driven candidate gene studies have not been particularly useful. Second, because we understand so little of the pathogenesis of schizophrenia, we have no biological gold standards or first principles. Put simply, there is neither a biology that we can demand of a '"true" associated gene nor a biology that is inconsistent with a "false" gene. Third, how then can we assess the validity of the biological connection being made? For genetics to achieve its goal of providing secure entry points into the biology of schizophrenia, findings must stand on their own merits without reference to other biological hypotheses or data. To do otherwise inevitably leads to circular reasoning (i.e., speculative biological supported by weak genetics supported by biological speculation).

Fourth, the criterion of biological salience is surprisingly inclusive. A large fraction of human genes are of legitimate interest to an integrative neuroscientist: depending on inclusion criteria, ⅓ to ⅔ of human genes are of biological interest (Figure 2). Genomic studies can test millions of hypotheses –hundreds of genetic markers will have *P*-values in the 10^{-5} to 10^{-7} range from the play of chance. These "intriguing" genetic markers will, merely by chance, often be located near a biologically "cool" gene. This is a meaningless coincidence until proven otherwise by genetic evidence.

Fifth, this argument is counter to best practices in human genetics that demand rigorous significance thresholds and replication. [51–55] For GWAS, the $5x10^{-8}$ threshold is nearly universally accepted. For rare variants of strong effect, a recent *Nature* paper summarized the recommendations of an NHGRI working group: [56] (a) "we emphasize the critical primacy of robust statistical genetic support for the implication of new genes, which may then be supplemented with ancillary experimental or informatic evidence supporting a mechanistic role"; (b) "Just as for genome-wide association studies of common variants, replication of newly implicated disease genes in independent families or population cohorts is critical supporting evidence, and in most cases essential for a novel gene to be regarded as convincingly implicated in disease"; and (c) "Without rigorous standards we risk an acceleration of false-positive reports of causality, which would impede the translation of genomic research findings into the clinical diagnostic setting and hinder biological understanding of disease".

Sixth, this argument is not accepted by many journals. *Nature Genetics* requires: "the genetic and statistical evidence for association should be sound. Molecular biological evidence for a functional variant is desirable in addition to, but will not substitute for, sound genetic evidence." [57] *PLoS Genetics* states: "genetic arguments should stand on their own". [58]

Finally, we note that invoking the biological argument is unnecessary. If statistical significance is marginal or if replication is required, then a more definitive study should be

designed and conducted in order to falsify the hypothesis. In many instances, this is achievable via collaboration and may be difficult for rarer genetic variants. We believe that schizophrenia genetics needs secure associations (significant beyond chance with precise replication) in order for genomic knowledge to be used as the essential anchor for understanding the biological basis of schizophrenia. Best practices in 2014 are thus very different from 2004. For example, the *AKT1* paper appeared in *Nature Genetics* in 2004 [59] with a considerable amount of biological and mouse model data but the genetic data are a SNP *P*-value of 0.05 (uncorrected for five genotyped SNPs) with no replication data.

Limitations - Can any historical candidate gene be formally excluded?

We have been careful to state that the current genomic evidence is inconsistent with an association for a particular gene. This is an evolving area, and it is possible that genes not now associated with schizophrenia will transition to significance with on-going expansions of sample sizes for common and rare variation. None of the historical candidate genes can be unequivocally excluded as a genetic risk factor for schizophrenia. However, we can state with high confidence that the large common variant genetic effects originally reported in many initial candidate gene studies are highly unlikely to be true.

Moreover, the location of some associations could provide a false clue, as genetic associations can act over long genomic distances – however, this assumption was made in the primary studies too. All current genomic technologies may miss some important types of genetic variation that play an etiological role.

It can also be argued that the genetic models used in the current generation of genomic studies for schizophrenia are inappropriate, that models should incorporate gene-environment, gene-gene, or even gene-gene-gene interactions. In a similar vein, it is possible that analyses that attempt to identify heterogeneity within the “schizophrenia” construct will prove informative. These hypotheses and alternative conceptualizations have merit and are now being investigated. Conducting these studies to a high standard is very difficult, and require unswerving adherence to accepted standards: thorough evaluation of bias, rigorous statistical significance thresholds, and replication are essential.

Conclusion

In summary, the current empirical evidence strongly supports the idea that the historical candidate gene literature yielded no robust and replicable insights into the etiology of schizophrenia. Even so, it is fair to note that these early studies unquestionably set the stage for the current era of genomic discovery for schizophrenia. These foundational efforts were a necessary step toward a better understanding of schizophrenia as a biological trait.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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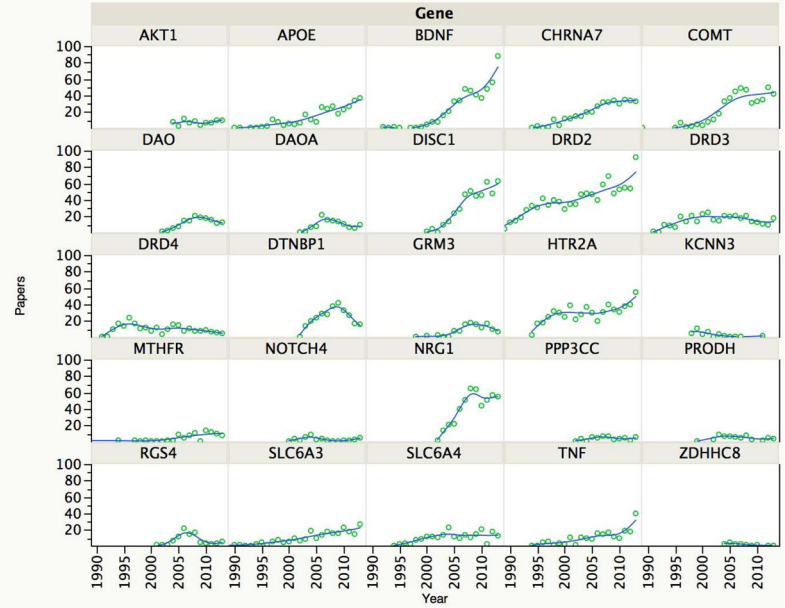


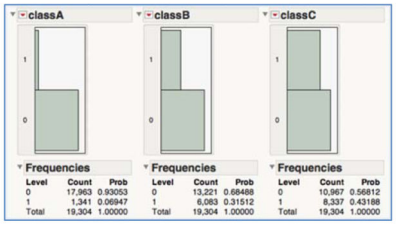
Figure 1.

Candidate gene publications per gene and per year. For each gene, the number of publications is indicated on the Y-axis, and the year is the X-axis. The data shown are from a PubMed query: (*gene* [All Fields] OR "*protein name*"[All Fields]) AND ("schizophrenia" [MeSH Terms] OR "schizophrenia"[All Fields]). The goal of this PubMed query was to provide a rough gauge of the impact of a candidate gene on the field (which differs from the "pre-GWAS" column in Table 1).

(A)

Class	Type	Genes
A	Genes implicated by GWAS for ADHD, autism, bipolar disorder, major depressive disorder, and schizophrenia [60]	825
	Genes with differential expression in post-mortem brain comparing bipolar disorder, major depression or schizophrenia to control [61]	279
	Genes in CNVs implicated in psychiatric disorders [62]	274
B	Autism genes & CNVs [63]	134
	Mental retardation genes [64-67]	501
	Genes with deleterious *de novo* exome sequence variation in autism or schizophrenia	1705
	Genes in CNVs implicated in developmental delay [68]	3163
	Genes whose proteins are at present at the synapse [69]	1033
	Genes whose proteins are part of the post-synaptic density [70]	720
	Genes whose mRNAs bind to FMRP [71]	831
C	Genes in OMIM for any disease [64]	3174
	Genes implicated by GWAS ($p < 5x10^{-9}$) for any trait (±10kb) [72]	1914
	Human orthologs of mouse genes whose knockout has a behavioral, neurological, or nervous system phenotype [73]	2983
	Genes implicated by psychiatric genome-wide linkage meta-analyses [74]	3308

(B)



classA	classB	classC	N Rows	% of Total
0	0	0	7455	38.62%
0	0	1	4980	25.80%
0	1	0	2862	14.83%
0	1	1	2666	13.81%
1	0	0	438	2.27%
1	0	1	348	1.80%
1	1	0	212	1.10%
1	1	1	343	1.78%

Figure 2. How many biologically interesting human genes are there?

This bioinformatic analysis addressed the question: how many human genes are of legitimate interest to an integrative neuroscientist or psychiatric geneticist? (A) We intersected 19,304 gene models from GENCODE (v17, “KNOWN” or “protein_coding”) with multiple data sources. Some genes can be in multiple categories. (B) Summary statistics (1=in set, 0=not in set): 35.6% of all genes are in classes A or B (=6869/19304), and 61.4% of all genes are in classes A, B, or C (=11849/19304). These numbers are conservative as adding “expression in brain at any developmental stage” would increase the numbers further. Thus, sizable proportions of all genes are of potential interest to a biologist. Biological interest is an imprecise criterion for the salience of a finding.

Table 1

Candidate genes of historical importance in schizophrenia research

Gene	Product	Reviews	Pre-GWAS	Rationale
AKT1	v-akt murine thymoma viral oncogene homolog 1	2	13	Mood disorder pharmacology [59]
APOE	Apolipoprotein E	1	32	Implicated in Alzheimer's disease [75]
BDNF	Brain-derived neurotrophic factor	0	40	Neurodevelopment hypothesis [76]
CHRNA7	Cholinergic receptor, nicotinic, α7	1	12	Linkage analysis [77]
COMT	Catechol-O-methyltransferase	4	81	22q11 CNV [78]
DAO	D-amino-acid oxidase	2	10	Linkage analysis, glutamate hypothesis [79]
DAOA	D-amino acid oxidase activator	3	27	Linkage analysis, glutamate hypothesis [79]
DISC1	Disrupted in schizophrenia 1	3	22	Translocation in a pedigree [80]
DRD2	Dopamine receptor D2	1	67	Antipsychotic pharmacology [81]
DRD3	Dopamine receptor D3	2	71	Dopamine hypothesis [20]
DRD4	Dopamine receptor D4	0	45	Antipsychotic pharmacology [82]
DTNBP1	Dystrobrevin binding protein 1	3	32	Linkage analysis [83]
GRM3	Glutamate receptor, metabotropic 3	1	15	Glutamate hypothesis [21]
HTR2A	Serotonin receptor 2A	2	57	Antipsychotic pharmacology [84]
KCNN3	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3	0	23	Discovery of a CAG repeat [85]
MTHFR	Methylenetetrahydrofolate reductase	0	20	Psychiatric symptoms with *MTHFR* dysfunction [86]
NOTCH4	Notch 4	0	24	Linkage analysis [87]
NRG1	Neuregulin 1	3	41	Linkage analysis [88]
PPP3CC	Protein phosphatase 3, catalytic subunit, γ isozyme	1	9	Linkage analysis/mouse phenotype [89]
PRODH	Proline dehydrogenase (oxidase) 1	3	10	22q11 CNV (incorrectly called "*PRODH2*" [90]
RGS4	Regulator of G-protein signaling 4	3	22	Differential expression in cases [91]
SLC6A3	Dopamine transporter	0	22	Dopamine hypothesis [92]
SLC6A4	Serotonin transporter	1	32	Implicated in mood disorders [93]
TNF	Tumor necrosis factor	0	21	Immune hypothesis [94]
ZDHHC8	Zinc finger, DHHC-type 8	2	9	22q11 CNV [95]

Reviews: the number of times a gene was in any of four selected reviews of schizophrenia genetics circa 2005. [10, 12–14] Pre-GWAS: the number of schizophrenia candidate gene papers studying this gene in calendar year 2008 or earlier. [2, 16] Rationale: the stated explanation for considering this gene as a candidate gene for schizophrenia according to the original publication. With the exception of *DISC1*, all studies evaluated common variant hypotheses.

Table 2

Empirical findings for 25 candidate genes.

Gene	Marker	SZGene OR (95% CI)	SZGene *P*	PGC OR (95% CI)	PGC *P*	PGC P_{min} (± 25kb)	Informed Investigator Rating ‡	Schizophrenia geneticists Rating ‡
AKT1	rs3730358	1.01 (0.91–1.13)	0.82	1.02 (0.99–1.06)	0.17	0.0003	5	2.5
APOE	ε.2/3/4	0.99 (0.82–1.20)	0.95	0.99 (0.96–1.02) †	0.48	0.0095		1.7
BDNF	270C/T	0.68 (0.52–0.87)	0.0028	1.01 (0.97–1.06) †	0.55	$8.0x10^{-5}$		3.0
	rs6265	0.95 (0.87–1.04)	0.29	0.95 (0.92–0.97)	$8.0x10^{-5}$			
CHRNA7	rs28531779	0.97 (0.72–1.30)	0.82	1.01 (0.96–1.05)	0.79	0.0096	5	2.9
COMT	rs4680	1.00 (0.96–1.05)	0.92	0.99 (0.97–1.01)	0.56	0.0065	1 §	2.4
DAO	rs3918346	1.00 (0.89–1.12)	0.94	1.03 (1.00–1.05)	0.035	0.0004	3	2.2
DAOA	rs3916965	0.95 (0.90–1.01)	0.11	1.00 (0.98–1.02)	0.96	0.015	3	2.0
DISC1	rs999710	1.07 (1.00–1.14)	0.045	1.01 (0.99–1.03)	0.29	0.00095	4.5	2.7
DRD2	rs1801028	0.85 (0.71–1.03)	0.10	0.95 (0.89–1.03)	0.22	$8.3x10^{-9}$	4	4.1
DRD3	rs6280	1.03 (0.97–1.08)	0.33	0.99 (0.97–1.01)	0.31	0.015	2	2.3
DRD4	rs4646983	1.13 (0.76–1.67)	0.56	No data	No data	0.0026		2.2
DTNBP1	rs3213207	1.10 (1.02–1.19)	0.015	1.04 (1.01–1.08)	0.012	0.0073	2	2.4
GRM3	rs2228595	1.21 (0.96–1.52)	0.099	1.01 (0.97–1.06)	0.58	$1.0x10^{-10}$		4.0
HTR2A	rs6311	1.14 (1.06–1.23)	0.0005	1.01 (0.99–1.04)	0.18	0.011	4	2.3
KCNN3	1333T/C	1.12 (0.33–3.76)	0.86	0.95 (0.93–0.98) †	$3.3x10^{-5}$	$6.8x10^{-6}$		3.0
MTHFR	rs1801133	1.09 (1.01–1.17)	0.026	1.01 (0.98–1.03)	0.55	0.016		2.1
NOTCH4	rs367398	1.00 (0.87–1.15)	0.99	No data	No data	$1.1x10^{-18}$		3.2
NRG1	rs62510682	0.94 (0.88–1.01)	0.074	0.97 (0.95–1.00)	0.024	0.0012	3	2.9
PPP3CC	rs7837713	0.99 (0.81–1.21)	0.91	1.01 (0.97–1.06)	0.62	0.00017		2.0
PRODH	rs383964	1.09 (0.88–1.35)	0.42	1.02 (0.97–1.07)	0.41	0.0092		2.0
RGS4	rs2661319	0.93 (0.88–0.99)	0.013	1.01 (0.99–1.03)	0.47	0.0061	2 ¶	2.1
SLC6A3	VNTR (rs28363170)	0.97 (0.82–1.16)	0.77	0.98 (0.94–1.01) †	0.24	0.0103		2.0
SLC6A4	5-HTTVNTR	1.11 (1.01–1.21)	0.024	0.91 (0.86–0.96) †	$4.2x10^{-4}$	0.00042		2.5

Gene	Marker	SZGene OR (95% CI)	SZGene *P*	PGC OR (95% CI)	PGC *P*	PGC P_{min} (± 25kb)	Informed Investigator Rating ‡	Schizophrenia geneticists Rating ‡
	5-HTTLPR	1.01 (0.94–1.09)	0.75	1.03 (1.00–1.07) †	0.058			
TNF	rs1800629	1.00 (0.86–1.17)	0.98	0.91 (0.89–0.94)	$5.6x10^{-10}$	$1.7x10^{-18}$		3.0
ZDHHC8	rs175174	1.00 (0.90–1.11)	0.96	0.98 (0.96–1.01)	0.17	$4.1x10^{-6}$		2.4

SZGene OR (odds ratio) and 95% CI (confidence interval) from our meta-analysis of SZGene [2]. Shown are the best marker per gene (full list in Table S1) plus two widely-studied markers (rs6265 and 5-HTTLPR). PGC OR and 95% CI from the PGC mega-analysis [11]. PGC *P*=*P*-value. PGC P_{min}=minimum *P*-value ±25 kb of a gene.

†For non-SNP markers, the smallest PGC *P*-value within 25 kb of a variant is shown. Shaded SZGene cells are nominally significant but far from genome-wide significance. Shaded PGC cells are genome-wide significant. Ratings that are ≥4 are shaded.

‡Raters were asked for a 1–5 ranking (1=very unlikely and 5=very likely): "What is your current summary judgment that genomic studies implicate *GENE* as a genetic risk factor for schizophrenia?" Supplemental Note provides detail. Schizophrenia geneticists ratings are means for N=24.

§Rating as a main effect, but "4" as an epistatic effect (Supplemental Note).

¶Rating as involved in the pathophysiology of schizophrenia would be "4" (Supplemental Note).